ISOZYME ANALYSIS OF SELECTED TAXA OF DWARF MISTLETOES: FINAL REPORT FOR PROJECT ENTITLES

Dr. Daniel L. Nickrent

FINAL REPORT

FOR PROJECT ENTITLED:

ISOZYME ANALYSIS OF SELECTED TAXA OF DWARF MISTLETOES

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Systematics and Population Biology of Two Sibling Species of *Arceuthobium* (Dwarf Mistletoes, Viscaceae)

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ABSTRACT: The systematic relationships and population biology of two closely related species of *Arceuthobium*, *A. campylopodum* and *A. occidentale*, were investigated using isozyme electrophoresis. In the study, ca. 500 individuals were examined across 16 populations and four host pine species. Although greater than 80% of the loci examined were polymorphic with a mean heterozygosity value of 0.22, nearly all genetic variation resides within populations. For all but one population, allelic frequencies for the majority of loci are in Hardy-Weinberg equilibrium. The one exception involves a population of mistletoes colonizing a secondary host species. Apportionment of variance using F-statistics did not indicate that host species is of any greater importance than other factors in shaping patterns of genetic variation. Interpopulational genetic similarity measures resulted in values of 90% or greater, except for the dwarf mistletoes collected from *Pinus radiata*. No isozyme marker alleles or consistent allelic frequency differences were noted between populations identified as *A. campylopodum* or *A. occidentale*.

INTRODUCTION: *Arceuthobium* M. VON BIEBERSTEIN (dwarf mistletoes, Viscaceae) is a well defined genus comprising ca. 40 Old and New World parasites (HAWKSWORTH and WIENS 1984). Trees in the family Pinaceae are hosts for dwarf mistletoes in the New World, whereas both Pinaceae and Cupressaceae are parasitized in the Old World. Since the early 1900's, at least four subgeneric classifications have been proposed (GILL 1935; KUIJT 1955; HAWKSWORTH and WIENS 1972, 1984; NICKRENT et al. 1984 and NICKRENT 1986). Although dwarf mistletoes are generally considered recalcitrant to taxonomic analysis, each successive study has utilized a broader database resulting in continual refinement of the classification of this group. To date, characters based upon morphology, flowering times, time of meiosis, pollen ultrastructure, secondary compound chemistry (flavonoids), host relationships, and isozymes have been used.

In the New World, the greatest number of dwarf mistletoe species occur in Mexico and the Sierra Nevada of the Western U.S. Nine species of *Arceuthobium* occur in the state of California, seven of which were placed in Section *Campylopodum* (HAWKSWORTH and WIENS 1972). NICKRENT (1986), using isozyme electrophoresis, examined 19

of the 32 New World taxa. From a systematic standpoint, several results emerged which are relevant to this study:

1. The genus as a whole exhibits high levels of genetic variation as compared with other dicots.

2. Cluster analysis of genetic distance measures resulted in grouping many taxa in accordance with the classification proposed by HAWKSWORTH and WIENS (1972). For those taxa that did not cluster as expected (such as *Arceuthobium divaricatum*), it became apparent that genetic affinity may be difficult to assess using traditional means. This result also emphasized that different classifications can result when different sources of data (e.g. morphology vs. isozymes) are utilized.

3. Isozyme analysis did not result in clearly demarcated species within Section *Campylopodum* as defined by HAWKSWORTH and WIENS (1972). Genetic similarity levels of 80% or greater were obtained across 11 taxa in this group.

These results emphasized the need for more intensive examination of the *Campylopodum* complex with the aim of defining species boundaries and illuminating the evolutionary processes associated with speciation. Two dwarf mistletoes, *Arceuthobium campylopodum* and *A. occidentale* were selected for further work. Several factors make these taxa ideal choices for study. First, they are quite similar as reflected by previous taxonomic treatments which considered them conspecific (ENGELMANN 1878, GILL 1935, KUIJT 1955) or very closely related (HAWKSWORTH and WIENS 1972, NICKRENT 1986). Second, the ranges of both taxa are contiguous and areas of sympatry can be found (Figure 1). Finally, although generally confined to different principal host species (*Pinus ponderosa* and *P. sabiniana*, respectively), each mistletoe can be found on the other's host species in certain areas.

Morphologically, *Arceuthobium campylopodum* and *A. occidentale* are remarkably similar. A feature used by HAWKSWORTH and WIENS (1972) to differentiate between them concerns the pre-flowering staminate spike length. For *A. campylopodum*, the spike is less than 10 mm long with a length/width ratio of 3 or less. For *A. occidentale*, the spikes are 10 mm or more long with a length/width ratio of 5 or more. Recently, HAWKSWORTH (per. com.) has questioned the validity of this distinguishing character. The range of shoot colors for both taxa overlaps, however *A. occidentale* is often yellow or orange and *A. campylopodum* is more often greenish. Phenologically, *A. campylopodum* flowers from August to October with a peak in mid to late August. *Arceuthobium occidentale* begins flowering in August and may continue into December with a peak in October. *A. occidentale* occurs at lower (hence warmer) elevations of the Sierras and Coastal ranges of California than does *A. campylopodum*. For at least four weeks, flowering times for these two taxa overlap, therefore it is theoretically possible that gene flow could occur when they occur sympatrically. No barriers to hybridization (e.g. interspecific incompatibility) have been reported and both taxa share the same chromosome number (X = 14).

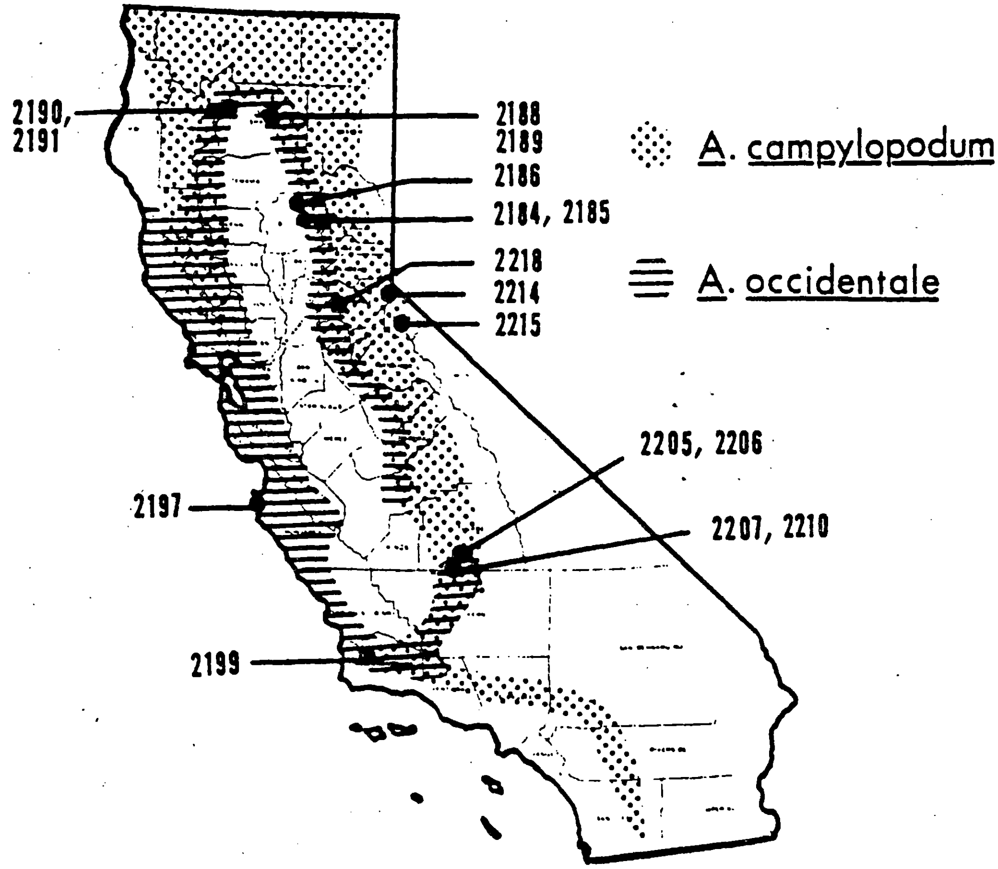

Figure 1. Distributions of Arceuthobium campylopodum and A. occidentale in California and locations of collection sites. See Table 2 for key to population numbers.

The natural host species for these two taxa are summarized in Table 1. It can be seen that only one host species, Pinus coulteri, serves as a primary host for both taxa and that each dwarf mistletoe is generally confined to different hosts. When a dwarf mistletoe is found on a secondary or rare host, the situation is termed a "cross-over". This more commonly occurs when only one mistletoe

TABLE 1. HOSTS OF ARCEUTHOBIUM CAMPYLOPODUM AND A. OCCIDENTALE

Dwarf Mistletoe	Primary Hosts	Secondary Hosts
A. campylopodum	Pinus ponderosa var. ponderosa	Pinus contorta (3 subspecies)
	Pinus jeffreyi	Pinus ponderosa scopulorum
	Pinus attenuata	Pinus sabiniana
	Pinus coulteri	
A. occidentale	Pinus sabiniana	P. attenuata
	P. muricata	P. contorta ssp. bolanderi
	P. coulteri	P. ponderosa
	P. radiata	P. jeffreyi

species is present in an area. The observation that secondary host infections rarely occur when the primary host/parasite combination is present has been termed competitive or host exclusion. On its primary hosts, _A. campylopodum_ infections induce branch proliferations called witches' brooms. On its primary host (_Pinus sabiniana_), _A. occidentale_ rarely induces such brooms. In cross-over situations, such as _A. campylopodum_ on _P. sabiniana_, brooms are not seen. This prompted HAWKSWORTH et al. (1985) to state that brooming response, at least for these two taxa, is related to which host the mistletoe is parasitizing.

The major objectives of this study were to 1) determine appropriate electrophoretic conditions for dwarf mistletoe shoot material, 2) assess whether isozyme markers exist which could be used to discriminate between the two species, and 3) determine whether patterns of genetic variability are best explained by mistletoe species, geographic locality, or host.

METHODS AND MATERIALS: Shoot material from eight populations each of _Arceuthobium campylopodum_ and _A. occidentale_ was collected within seven counties in California during the fall of 1986 (Table 2). Individual samples (ca. 5 g) were ground to a powder with a mortar and pestle using liquid nitrogen and then homogenized in an extraction buffer reported by FERET (1971) as modified by PITEL and CHELIAK (1984) with 10% w/v Polyvinylpyrrolidone (PVP-40). The extract was then centrifuged at 10,000 rpm for 15 minutes and the supernatant stored frozen (-70° C) in 1.5 ml microcentrifuge tubes. Extracts were kept frozen until ready to load on the gel. Enzyme separation was accomplished using 14% starch gels as reported by SHAW and PRASAD (1970).

TABLE 2. _ARCEUTHOBIUM_ POPULATIONS SAMPLED FOR ELECTROPHORESIS

Pop. No.		_Arceuthobium_ Species	_Pinus_ Host	Population Name, and California County
1.	2184	_A. occidentale_	_P. sabiniana_	Paradise-1, Butte Co.
2.	2185	_A. occidentale_	_P. sabiniana_	Paradise-2, Butte Co.
3.	2186	_A. occidentale_	_P. sabiniana_	Chico, Butte Co.
4.	2188	_A. campylopodum_	_P. ponderosa_	Lassen-1, Shásta Co.
5.	2189	_A. campylopodum_	_P. ponderosa_	Lassen-2, Shasta Co.
6.	2190	_A. occidentale_	_P. sabiniana_	Manton, Shasta Co.
7.	2191	_A. campylopodum_	_P. ponderosa_	Manton, Shasta Co.
8.	2197	_A. occidentale_	_P. radiata_	Monterey, Monterey Co.
9.	2199	_A. occidentale_	_P. sabiniana_	Figueroa, Santa Barbara Co.
10.	2205	_A. campylopodum_	_P. jeffreyi_	Poison Meadow-1, Tulare Co.
11.	2206	_A. campylopodum_	_P. sabiniana_	Poison Meadow-2, Tulare Co.
12.	2207	_A. occidentale_	_P. sabiniana_	Kern River-5, Tulare Co.
13.	2210	_A. campylopodum_	_P. ponderosa_	Greenhorn, Tulare Co.
14.	2214	_A. campylopodum_	_P. jeffreyi_	Emerald Bay, El Dorado Co.
15.	2215	_A. campylopodum_	_P. jeffreyi_	Indian Creek, Alpine Co.
16.	2218	_A. occidentale_	_P. sabiniana_	Placerville, El Dorado Co.

The following 9 enzyme systems were used for this study: Adenylate Kinase (AK, E.C. 2.7.4.3), Alcohol Dehydrogenase (ADH, E.C. 1.1.1.1), Fluorescent Esterase (F-EST, 3.1.1.-), Glucose-6-Phosphate Dehydrogenase (G-6-PDH, 1.1.1.49), Isocitrate Dehydrogenase (IDH, 1.1.1.42), Malate Dehydrogenase (MDH, 1.1.1.37), Phosphoglucoisomerase (PGI, 5.3.1.9), Phosphoglucomutase (PGM, 2.7.5.1), and 6-Phosphogluconate Dehydrogenase (6-PGDH, 1.1.1.44). All systems displayed a single scorable locus except MDH and ADH where two loci were used. Additional enzyme systems obtained from dwarf mistletoe tissue but not reported here include Glutamate Pyruvate Transaminase, Glutathione Reductase, Leucine Amino Peptidase, Peroxidase, and Triose Phosphate Isomerase. For AK, G-6-PDH, IDH, PGI, PGM, and 6-PGDH, the Tris-citrate buffer, pH 7.5 reported in SOLTIS et al. (1983) was used. For ADH, F-EST, and MDH, the citrate-morpholine buffer reported by CLAYTON and TRETIAK (1972) as modified by NICKRENT (1986) was used. Enzyme staining was as reported in NICKRENT (1986).

Genotypic loci were inferred directly from electrophoretic phenotypes and should therefore be considered putative since no crosses and genetic analyses were used to document inheritance patterns. Such crosses are possible but not readily accomplished given the time from seed to seed (2 to 6 years) in these parasites. Banding patterns for Arceuthobium were readily interpretable, however, because 1) they conform to expected patterns reported for other diploid plants in terms of enzyme quaternary structure (GOTTLIEB 1982) and 2) the number of loci per enzyme system was low. It should be noted that several enzyme systems usually represented by a cytosolic and plastid locus (such as 6-PGDH and PGI) in higher plants are present as only one scorable (presumably cytosolic) locus in these mistletoes. The migration distance of the most common band present at each locus was assigned the relative mobility "100" and additional bands were given numbers as percentages of it. The relative mobility designations were translated to genotype data (e.g. AA, AB, BB, AC, etc.) for each individual per population and these were analyzed for genetic variability using the computer program BIOSYS-1 (SWOFFORD and SELANDER 1981).

RESULTS: Given that a polymorphic locus is one for which the frequency of the most common allele does not exceed 0.99, then all loci examined in this study are polymorphic across the 16 populations. Depending on the locus, between 300 and 500 individuals were analyzed for this study. A total of 76 alleles for the 11 loci were seen following analysis of all populations (Table 3). 6-PGDH was the most diverse locus with ten alleles. Table 3 shows the allele frequencies for each enzyme system and population and Table 4 summarizes the genetic variability measurements for all populations.

The mean number of alleles per locus for all populations of A. campylopodum and A. occidentale are 3.07 and 2.58 respectively. These values are similar to those reported by NICKRENT (1986) for these two taxa (2.37 and 2.2 respectively) where triploid seed endosperm was used for the isozyme analysis. In the present study, mean values for percent polymorphic loci are 89.7 and 79.5 for these two taxa. The previous analysis of endosperm gave 78.8 for A. campylopodum and 81.8 for A. occidentale, however only one

TABLE 3. ALLELIC FREQUENCIES FOR ELEVEN LOCI IN *ARCEUTHOBIUM*

	POPULATION															
LOCUS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ADH-1																
(N)	14	12	43	32	31	28	20	47	66	34	59	13	8	56	36	9
A	0.929	1.000	0.930	0.906	0.839	0.821	0.900	0.861	0.833	0.926	0.967	0.847	0.875	0.866	0.986	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.125	0.000	0.014	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.038	0.000	0.009	0.000	0.000
E	0.000	0.000	0.058	0.094	0.161	0.036	0.075	0.074	0.000	0.074	0.008	0.000	0.000	0.107	0.000	0.000
F	0.071	0.000	0.012	0.000	0.000	0.143	0.025	0.032	0.167	0.000	0.008	0.115	0.000	0.018	0.000	0.000
ADH-2																
(N)	14	12	43	32	31	28	20	47	66	35	58	13	8	56	37	9
A	0.429	0.708	0.779	0.781	0.742	0.518	0.500	0.223	0.758	0.729	0.750	0.885	0.812	0.518	0.608	0.722
B	0.571	0.292	0.221	0.219	0.258	0.464	0.475	0.777	0.242	0.271	0.250	0.115	0.125	0.482	0.392	0.278
C	0.000	0.000	0.000	0.000	0.000	0.018	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.000
AK																
(N)	14	12	43	32	31	1	1	48	66	35	59	13	8	10	2	9
A	0.857	1.000	0.988	1.000	1.000	1.000	1.000	1.000	1.000	0.972	0.967	1.000	1.000	0.950	1.000	1.000
B	0.143	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.025	0.000	0.000	0.050	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.008	0.000	0.000	0.000	0.000	0.000
F-EST																
(N)	14	12	40	32	30	23	17	43	36	35	59	12	8	42	21	9
A	0.750	0.916	0.900	0.969	0.983	0.760	0.794	0.674	0.708	0.929	0.975	0.958	0.750	0.076	0.881	0.778
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.042	0.063	0.031	0.017	0.174	0.147	0.174	0.000	0.043	0.008	0.000	0.125	0.012	0.071	0.111
D	0.107	0.000	0.037	0.000	0.000	0.022	0.000	0.140	0.264	0.029	0.017	0.042	0.125	0.012	0.000	0.111
E	0.143	0.042	0.000	0.000	0.000	0.022	0.000	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.024	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000
G	0.000	0.000	0.000	0.000	0.000	0.022	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
G-6-PDH																
(N)	14	12	43	27	30	24	17	43	66	35	56	13	8	51	27	9
A	1.000	1.000	0.837	0.722	0.867	0.979	0.912	0.791	0.818	1.000	0.982	1.000	0.812	0.892	0.777	0.889
B	0.000	0.000	0.000	0.000	0.017	0.021	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.163	0.222	0.083	0.000	0.029	0.209	0.167	0.000	0.018	0.000	0.188	0.059	0.204	0.000
D	0.000	0.000	0.000	0.056	0.033	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.049	0.019	0.111
IDH																
(N)	14	12	43	32	31	28	20	48	66	35	58	13	8	56	36	9
A	0.821	0.958	0.919	0.734	0.952	0.660	0.800	1.000	0.954	0.713	0.802	0.846	0.812	0.839	0.903	1.000
B	0.000	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000
C	0.107	0.000	0.058	0.094	0.016	0.250	0.100	0.000	0.015	0.100	0.172	0.154	0.000	0.027	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.086	0.009	0.000	0.188	0.018	0.014	0.000
E	0.000	0.000	0.000	0.016	0.000	0.054	0.025	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000
F	0.036	0.000	0.023	0.109	0.016	0.036	0.075	0.000	0.008	0.029	0.000	0.000	0.000	0.098	0.083	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.018	0.000	0.000
H	0.036	0.042	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 3. CONTINUED

LOCUS	POPULATION 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
MDH-3																
(N)	14	12	43	32	31	28	20	47	66	35	59	13	8	56	37	9
A	0.064	0.958	0.860	0.859	0.936	0.893	0.950	0.490	0.591	0.786	0.703	0.731	0.875	0.884	0.878	0.833
B	0.000	0.000	0.012	0.000	0.016	0.000	0.000	0.000	0.045	0.029	0.035	0.000	0.000	0.000	0.000	0.000
C	0.036	0.042	0.128	0.141	0.048	0.107	0.050	0.170	0.364	0.071	0.051	0.231	0.125	0.116	0.095	0.167
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.340	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.114	0.076	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.127	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
MDH-4																
(N)	14	12	40	32	31	28	20	48	66	34	59	13	8	53	37	9
A	1.000	1.000	0.988	0.906	0.968	1.000	0.975	1.000	0.970	0.956	0.924	0.847	1.000	1.000	1.000	0.944
B	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.056
D	0.000	0.000	0.000	0.000	0.032	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.051	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.000	0.030	0.044	0.000	0.115	0.000	0.000	0.000	0.000
G	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGI																
(N)	13	12	43	32	30	28	20	47	66	35	59	13	8	56	37	9
A	0.654	0.833	0.662	0.687	0.750	0.750	0.725	0.851	0.765	0.900	0.949	0.731	0.874	0.651	0.797	0.611
B	0.308	0.167	0.232	0.297	0.166	0.196	0.225	0.128	0.152	0.100	0.017	0.269	0.063	0.313	0.149	0.389
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.027	0.000
D	0.000	0.000	0.047	0.000	0.017	0.018	0.025	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.034	0.000	0.000	0.036	0.027	0.000
G	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.038	0.000	0.035	0.016	0.067	0.036	0.025	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
PGM																
(N)	6	10	9	31	29	11	12	48	65	35	45	11	8	48	37	6
A	0.666	0.800	1.000	0.710	0.708	0.864	0.708	0.979	0.538	0.742	0.678	0.500	0.812	0.376	0.728	0.584
B	0.000	0.000	0.000	0.016	0.017	0.000	0.042	0.000	0.062	0.000	0.033	0.136	0.063	0.052	0.014	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.010	0.014	0.000
D	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.050	0.000	0.129	0.034	0.091	0.000	0.021	0.238	0.143	0.000	0.000	0.000	0.396	0.095	0.083
F	0.000	0.000	0.000	0.016	0.086	0.045	0.250	0.000	0.062	0.029	0.067	0.364	0.125	0.083	0.149	0.000
G	0.167	0.150	0.000	0.129	0.155	0.000	0.000	0.000	0.046	0.086	0.089	0.000	0.000	0.031	0.000	0.333
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038	0.000	0.133	0.000	0.000	0.000	0.000	0.000
I	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.000	0.000
6-PGDH																
(N)	14	12	42	32	31	28	20	48	66	35	59	13	8	56	37	9
A	0.714	0.583	0.857	0.766	0.727	0.660	0.825	0.354	0.871	0.643	0.636	0.462	0.750	0.517	0.595	0.888
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.085	0.077	0.000	0.000	0.054	0.000
C	0.000	0.000	0.024	0.031	0.000	0.018	0.000	0.083	0.000	0.000	0.008	0.000	0.000	0.045	0.000	0.056
D	0.000	0.000	0.036	0.063	0.048	0.125	0.025	0.000	0.045	0.071	0.093	0.115	0.063	0.027	0.027	0.056
E	0.179	0.417	0.083	0.108	0.177	0.036	0.000	0.438	0.053	0.243	0.153	0.308	0.187	0.312	0.324	0.000
F	0.000	0.000	0.000	0.000	0.016	0.161	0.150	0.021	0.008	0.000	0.000	0.000	0.000	0.063	0.000	0.000
G	0.000	0.000	0.000	0.016	0.000	0.000	0.000	0.104	0.023	0.000	0.000	0.038	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.000	0.000
I	0.107	0.000	0.000	0.016	0.032	0.000	0.000	0.000	0.000	0.014	0.025	0.000	0.000	0.000	0.000	0.000
J	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.000

TABLE 4. GENETIC VARIABILITY IN 16 ARCEUTHOBIUM POPULATIONS

Pop. No.	Mean Sample Size/Locus	Mean Number Alleles/Locus	% Loci Polymorphic	Mean Heterozygosity Direct Count	Mean Heterozygosity H-W Expected
2184	13.2	2.4	81.8	0.224	0.293
2185	11.8	1.8	63.6	0.192	0.174
2186	39.3	2.8	90.9	0.165	0.196
2188	31.5	3.1	90.9	0.284	0.291
2189	30.5	3.1	90.9	0.203	0.233
2190	23.2	3.0	81.8	0.244	0.288
2191	17.0	2.8	90.9	0.209	0.267
2197	46.7	2.7	72.7	0.218	0.276
2199	63.2	3.5	90.9	0.253	0.303
2205	34.8	3.1	90.9	0.229	0.255
2206	57.3	3.8	100.0	0.198	0.248
2207	12.7	2.5	81.8	0.275	0.299
2210	8.0	2.3	81.8	0.227	0.264
2214	49.1	3.5	90.9	0.262	0.309
2215	31.3	2.9	81.8	0.247	0.257
2218	8.7	2.0	72.7	0.202	0.249
Means	29.9	2.8	84.6	0.226	0.262

population of the latter species was sampled. Mean heterozygosity for *A. campylopodum* is slightly higher (0.232) than that of *A. occidentale* (0.221).

Analyses were conducted to determine whether each population was in Hardy-Weinberg equilibrium at each variable locus (tables not reproduced here). This operation was performed using observed genotype frequencies and those expected under Hardy-Weinberg equilibrium and conducting a chi-square goodness-of-fit test. When more than two alleles occur at a locus (as is the case for many *Arceuthobium* loci), BIOSYS-1 pools genotypes into three classes: homozygotes for the most common allele, heterozygotes for the most common allele and one other allele, and all other genotypes. For 13 of the 16 populations, one or two loci (but not always the same ones) showed significant deviation from Hardy-Weinberg equilibrium. Population 2206, however, showed deviation at ADH-1, IDH, MDH-3, PGI, and PGM, i.e. for more loci than any other population. It is important to note that these plants were identified as *Arceuthobium campylopodum* and were growing on *Pinus sabiniana* in a cross-over situation. Population 2205 represents *A. campylopodum* individuals occurring sympatrically with population 2206, but on *P. jeffreyi*, a primary host. For this population, only PGM showed significant deviation.

For all populations, the Fixation Index F (WRIGHT 1969) was employed to measure deviation of heterozygote proportions from those expected under Hardy-Weinberg equilibrium. The value F can range

from -1.0 (excess heterozygotes) to 1.0 (deficiency of heterozygotes). Table 5 shows results of analysis of one population (2206) illustrating heterozygote deficiency for at least six of the 11 polymorphic loci.

TABLE 5. FIXATION INDEX* FOR _A. CAMPYLOPODUM_ (POP. 2206)

Locus	Observed Heterozygotes	Expected Heterozygotes	Fixation Index (F)
ADH-1	2	3.94	0.488**
ADH-2	23	21.94	- 0.057
AK	4	3.92	- 0.028
F-EST	3	2.97	- 0.020
G-6-PDH	0	1.98	1.000**
IDH	12	19.14	0.368**
MDH-3	26	28.53	0.081
MDH-4	6	8.56	0.293**
PGI	4	5.81	0.306**
PGM	13	23.18	0.433**
6-PGDH	31	33.09	0.055

* WRIGHT (1969)

** Heterozygote proportions significantly different than those expected under Hardy-Weinberg equilibrium.

The apportionment of genetic diversity within and among populations of _Arceuthobium campylopodum_ and _A. occidentale_ was examined using F-statistics (WRIGHT 1978). The formula employed by BIOSYS-1 is as follows:

$$1 - F_{IT} = (1 - F_{IS})\ (1 - F_{ST})$$

where F_{IT} = the fixation index of individuals relative to all populations

F_{IS} = the fixation index of individuals relative to their specific population

F_{ST} = measures the differentiation between populations relative to the limiting amount under complete fixation

The means of F_{IS}, F_{IT}, and F_{ST} across all populations and loci are shown in Table 6. F_{ST} values can range from 1.0 (all genetic diversity residing between populations) to 0 (all diversity within individuals of a population). The mean value for F_{ST} of 0.093 indicates that differentiation between populations is quite limited. This follows from the observation that most populations share a significant number of the same alleles and vary only in their frequencies. When comparing any of the populations, fixation for alternate alleles at a locus has not occurred.

TABLE 6. SUMMARY OF F-STATISTICS FOR ALL LOCI AND POPULATIONS

Locus	F_{IS}	F_{IT}	F_{ST}
ADH-1	0.021	0.075	0.055
ADH-2	0.125	0.235	0.125
AK	- 0.101	- 0.016	0.078
F-EST	0.127	0.199	0.082
G-6-PDH	0.403	0.454	0.087
IDH	0.080	0.150	0.076
MDH-3	- 0.049	0.064	0.108
MDH-4	0.165	0.209	0.053
PGI	0.024	0.074	0.052
PGM	0.273	0.363	0.124
6-PGDH	0.056	0.145	0.094
Means	0.112	0.195	0.093

By combining F-statistics with a hierarchical analysis, it can be determined whether populational differentiation has occurred with respect to the various levels of the hierarchy. For this analysis, the levels are SPECIES (*Arceuthobium campylopodum* and *A. occidentale*), HOST (*Pinus ponderosa*, *P. jeffreyi*, *P. sabiniana*, and *P. radiata*), and POPULATION (2184 through 2218 - Table 2). Table 7 summarizes the variance components and F-statistics summed across all 16 loci. The first three categories showing negative variance components indicate that each interaction tested does not significantly influence partitioning of genetic variation. The levels of comparison were the host with the dwarf mistletoe species associated with it, the host with all parasite populations totaled, and the two dwarf mistletoe species categories (*A. campylopodum* vs. *A. occidentale*) compared with all populations.

Positive variance components were obtained for the next three comparisons between hierarchical levels. The variance attributable to the interaction between populations and the total, populations and dwarf mistletoe species, and populations and hosts are nearly

TABLE 7. VARIANCE COMPONENTS AND F-STATISTICS COMBINED ACROSS ALL LOCI FOR *ARCEUTHOBIUM CAMPYLOPODUM* AND *A. OCCIDENTALE*

COMPARISON X	with	Y	VARIANCE COMPONENT	F_{XY}
HOST		MISTLETOE SPECIES	- 0.056	- 0.018
HOST		TOTAL POPULATIONS	- 0.070	- 0.023
MISTLETOE SPECIES		TOTAL POPULATIONS	- 0.013	- 0.004
MISTLETOE POPULATION		TOTAL POPULATIONS	0.218	0.070
MISTLETOE POPULATION		MISTLETOE SPECIES	0.232	0.074
MISTLETOE POPULATION		HOST	0.288	0.091

equal (from 0.218 to 0.288). These results indicate that as much variation exists between the two dwarf mistletoe species categories as between any of the separate populations.

The unbiased genetic identity measure of NEI (1978) and the genetic similarity measure of ROGERS (1972) were calculated for all pairwise comparisons of dwarf mistletoe populations. The matrix of these values is shown in Table 8 (next page), with the measure of NEI above the diagonal and ROGERS below. The most striking result is that all populations, using the NEI measure, are similar at the 90% level or above. To graphically depict interpopulational genetic identity values, a hierarchical cluster analysis using the unweighted pair group method with arithmetic averaging (UPGMA) algorithm (SNEATH and SOKAL 1973) was performed. Cluster analysis was performed on several similarity or distance measures, however the measure of NEI had the lowest percent standard deviation (FITCH and MARGOLIASH 1967) and the highest cophenetic correlation (0.937 and 0.917, respectively). The resulting phenogram is shown in Figure 2. The phenogram did not result in discrete clusters

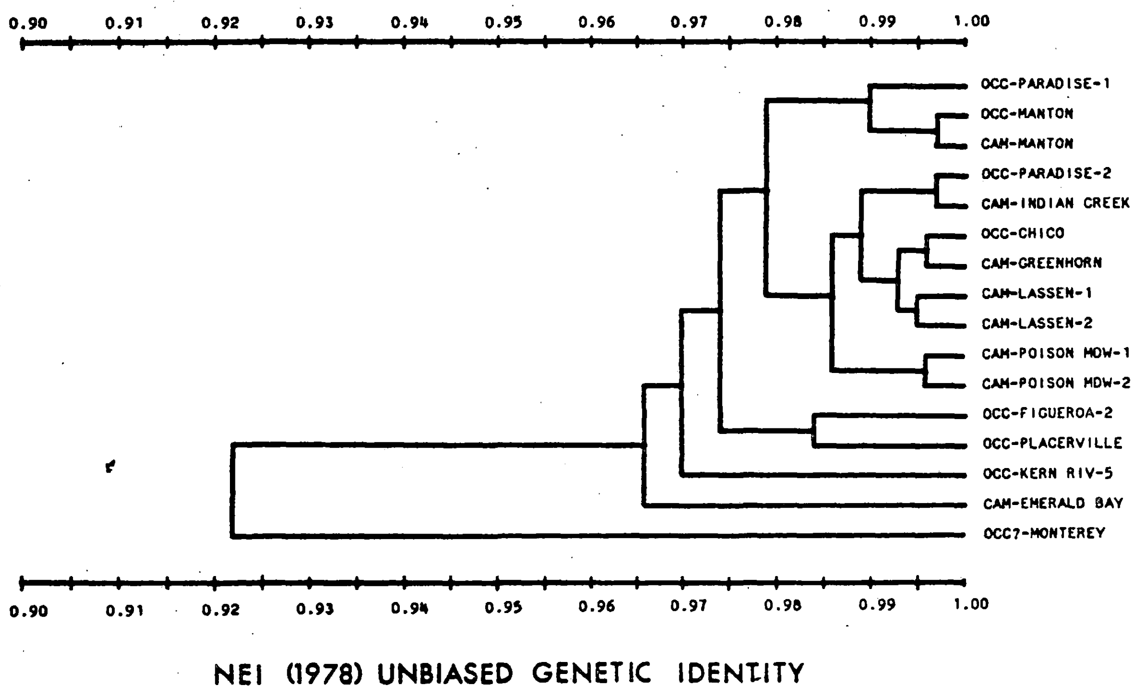

Figure 2. UPGMA phenogram for 16 dwarf mistletoe populations using NEI (1978) unbiased genetic identity. See Table 2 for key to population names. Putative species identifications are shown as CAM (<u>Arceuthobium campylopodum</u>) and OCC (<u>A. occidentale</u>).

composed entirely of populations of one species or the other. The population interpreted as <u>A. occidentale</u> from Monterey, California appears most dissimilar and joins all other populations at the 92% level.

TABLE 8. NEI GENETIC IDENTITY AND ROGERS GENETIC SIMILARITY COEFFICIENTS BETWEEN 16 ARCEUTHOBIUM POPULATIONS*

POP.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.	*****	0.988	0.973	0.972	0.982	0.988	0.992	0.939	0.955	0.978	0.968	0.951	0.969	0.976	0.982	0.987
2.	0.881	*****	0.984	0.984	0.996	0.978	0.979	0.940	0.960	0.996	0.988	0.980	0.993	0.977	0.997	0.983
3.	0.859	0.896	*****	0.993	0.991	0.980	0.983	0.921	0.971	0.982	0.975	0.962	0.996	0.950	0.984	0.987
4.	0.855	0.880	0.926	*****	0.995	0.975	0.981	0.906	0.973	0.987	0.978	0.973	0.992	0.970	0.987	0.991
5.	0.871	0.925	0.920	0.922	*****	0.978	0.987	0.920	0.971	0.991	0.986	0.979	0.995	0.975	0.992	0.991
6.	0.884	0.876	0.880	0.869	0.877	*****	0.997	0.936	0.956	0.984	0.974	0.956	0.980	0.962	0.978	0.972
7.	0.895	0.878	0.893	0.883	0.900	0.926	*****	0.928	0.962	0.980	0.974	0.962	0.984	0.969	0.986	0.985
8.	0.801	0.829	0.825	0.787	0.809	0.822	0.811	*****	0.907	0.927	0.918	0.895	0.924	0.920	0.951	0.906
9.	0.824	0.847	0.888	0.875	0.884	0.844	0.850	0.810	*****	0.967	0.964	0.963	0.982	0.953	0.967	0.984
10.	0.865	0.923	0.892	0.907	0.912	0.886	0.877	0.807	0.858	*****	0.996	0.980	0.995	0.972	0.989	0.977
11.	0.852	0.899	0.878	0.885	0.901	0.863	0.863	0.788	0.851	0.935	*****	0.980	0.988	0.957	0.982	0.972
12.	0.827	0.865	0.851	0.868	0.866	0.841	0.846	0.772	0.846	0.877	0.880	*****	0.979	0.964	0.975	0.966
13.	0.837	0.880	0.908	0.892	0.898	0.872	0.872	0.824	0.876	0.893	0.876	0.845	*****	0.954	0.995	0.983
14.	0.877	0.875	0.861	0.880	0.888	0.869	0.883	0.805	0.835	0.874	0.851	0.857	0.835	*****	0.981	0.968
15.	0.867	0.928	0.905	0.899	0.912	0.885	0.899	0.851	0.866	0.903	0.883	0.856	0.906	0.897	*****	0.977
16.	0.860	0.884	0.899	0.890	0.893	0.850	0.874	0.782	0.881	0.865	0.858	0.836	0.863	0.853	0.869	*****

* NEI (1978) unbiased genetic identity above diagonal, ROGERS (1972) genetic similarity below diagonal.

DISCUSSION: The results presented above are in agreement with the previous isozyme study by NICKRENT (1986). _Arceuthobium campylopodum_ and _A. occidentale_ show high levels of genetic similarity which circumvents differentiation into established species categories by electrophoretic means. These plants exhibit considerable genetic variation, however most is partitioned among individuals of populations, not between populations. F-statistic analyses indicate that the component attributable to host provides an equal influence on the distribution of genetic variation in populations as other components (e.g. mistletoe species).

Nearly all of the populations sampled were in Hardy-Weinberg equilibrium for the majority of isozyme loci examined. As a dioecious plant, _Arceuthobium_ is an obligate outcrosser and pollination is mediated by (primarily) insects and wind (PENFIELD et al. 1976). Gene flow within populations has not been measured directly in dwarf mistletoes, however the results presented here suggest that mating is random and that genetic drift, migration, selection, and other forces have not resulted in fixation for different alleles in different populations. Population 2206 showed significant deviation from Hardy-Weinberg equilibrium as well as heterozygote deficiencies. At this site (populations Poison Meadow-1 and 2), _A. campylopodum_ was collected from two different hosts, _Pinus jeffreyi_ (2205), a primary host, and _P. sabiniana_ (2206), a rare host combination (cross-over). Greater than fifty mistletoes were collected from each host tree, hence sample sizes appear adequate to assess genotype frequencies. In Figure 2, these populations cluster together at the 99% similarity level. Since they share the majority of electrophoretically detectable alleles and at similar frequencies, a reasonable conclusion is that both collections represent the same species. The deviation from Hardy-Weinberg equilibrium in the mistletoes collected from _P. sabiniana_ may reflect the results of selection against seedling genotypes that were not able to become established on this alternate host. If this is the case, this is evidence of genetic differentiation along host lines in these dwarf mistletoes.

The population of mistletoes at Manton were also parasitizing two hosts, _Pinus sabiniana_ (2190) and _P. ponderosa_ (2191). At this site it was difficult to determine morphologically whether one or two parasite species were present. Plants were assigned to _A. campylopodum_ or _A. occidentale_ depending upon which host was being parasitized. The phenogram in Fig. 2 illustrates the high degree of genetic identity among these plants, despite being collected from different host species. This example highlights the difficulty in choosing between two hypotheses. One possibility is that this situation represents one parasite taxon on two hosts (its primary and a cross-over host). Conversely, two sympatric, sibling species could exist here, each parasitizing its own primary host tree.

The results of isozyme analysis showed that the population of dwarf mistletoe from Monterey, California (2197) has undergone genetic differentiation with respect to the other populations examined. HAWKSWORTH and WIENS (1972) and HAWKSWORTH et al. (1984) placed this taxon within _Arceuthobium occidentale_. While conducting field collections, several features of these plants were noted that differed from "typical" _A. occidentale_. The shoot color was olive

green instead of orange/yellow, seed dispersal was underway during the first week in October, and the majority of Monterey pines were showing non-systemic brooms as a result of this parasite. Whether this taxon is A. occidentale, A. campylopodum, neither, or both remains to be determined.

HAWKSWORTH et al. (1984) mention other populations of A. campylopodum, such as those parasitizing Pinus attenuata (knobcone) and P. contorta ssp. contorta (shore pine) in the Gasquet area of northern California, that may represent additional genetically distinct elements in the species complex. To adequately measure levels of genetic variation in A. campylopodum and A. occidentale, further sampling is necessary from such populations occurring on secondary hosts. These parasites merit further study for they may provide an avenue to study recent evolutionary adaptation and incipient speciation events.

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REFERENCES:

Clayton, J. W. and Tretiak, D. N. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. Journ. Fish. Res. Board Canada 29: 1169-1172.

Engelmann, G. 1878. Loranthaceae. Report upon U.S. Geographical surveys west of the 100th. meridian (Wheeler Report). 6: (Botany): 251-254.

Feret, P. P. 1971. Isoenzyme variation in Picea glauca (Moench) Voss seedlings. Silvae Genet. 20: 46-50.

Fitch, W. M. and Margoliash, E. 1967. Construction of phylogenetic trees. Science 155: 279-284.

Gill, L. S. 1935. Arceuthobium in the United States. Conn. Acad. Arts and Sci. Trans. 32: 111-245.

Gottlieb, L. D. 1982. Conservation and duplication of isozymes in plants. Science 216: 373-380.

Hawksworth, F. G., Scharpf, R. F., and Mark, W. R. 1984. Taxonomic status of the Arceuthobium campylopodum - A. occidentale complex. Unpublished manuscript.

Hawksworth, F. G. and Wiens, D. 1972. Biology and classification of dwarf mistletoe (Arceuthobium). U.S.D.A. For. Ser. Agric. Handbook No. 401. 234 pp.

Hawksworth, F. G. and Wiens, D. 1984. Biology and classification of *Arceuthobium*: an update. *In*: Biology of Dwarf Mistletoes: Proceedings of the Symposium. USDA For. Ser. Gen. Tech. Rep. RM-111, eds. F. G. Hawksworth and R. F. Sharpf. pp. 2-17.

Kuijt, J. 1955. Dwarf mistletoes. Bot. Rev. 1: 569-627.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Human Genet. 41: 225-233.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Nickrent, D. L., Guttman, S. I., and Eshbaugh, W. H. 1984. Biosystematic and evolutionary relationships among selected taxa of New World *Arceuthobium*. *In*: Biology of Dwarf Mistletoes: Proceedings of the Symposium. USDA For. Ser. Gen. Tech. Rep. RM-111, eds. F. G. Hawksworth and R. F. Sharpf. pp. 20-35.

Nickrent, D. L. 1986. Genetic polymorphism in the morphologically reduced dwarf mistletoes (*Arceuthobium*, Viscaceae): an electrophoretic study. Amer. J. Bot. 73: 1492-1502.

Penfield, F. B., Stevens, R. E., and Hawksworth, F. G. 1976. Pollination ecology of three Rocky Mountain dwarf mistletoes. For. Sci. 22: 473-484.

Pitel, J. A., and Cheliak, W. M. 1984. Effect of extraction buffers on characterization of isoenzymes from vegetative tissues of five conifer species: a users manual. Can. For. Ser. Inform. Rept. PI-X-34, Petawawa National Forestry Institute. 64 pp.

Rogers, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics, Univ. Texas Pub. 7713: 145-153.

Shaw, C. R., and Prasad, R. 1970. Starch gel electrophoresis of enzymes-a compilation of recipes. Biochem. Gen. 4: 297-320.

Sneath, P. H. A., and Sokal, R. R. 1973. Numerical Taxonomy. W. H. Freeman, San Francisco.

Soltis, D. E., Haufler, C. H., Darrow, D. C., and Gastony, G. J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer. Fern Jour. 73: 9-27.

Swofford, D. L., and Selander, R. B. 1981. BIOSYS-I: a fortran computer program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journ. Hered. 72: 281-283.

Wright, S. 1969. Evolution and genetics of populations, Vol. 2. University of Chicago Press, Chicago.

Wright, S. 1978. Evolution and genetics of populations, Vol. 4. University of Chicago Press, Chicago.